PATENT

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PROVISIONAL PATENT APPLICATION

for

CANCER TREATMENT USING THERAPEUTIC CONJUGATES THAT BIND TO AMINOPHOSPHOLIPIDS

by

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WHAT IS CLAIMED IS:

1. A method for delivering a selected therapeutic agent to intratumoral vasculature, comprising administering to an animal having a vascularized tumor a biologically effective amount of a binding ligand that comprises said selected therapeutic agent operatively attached to a targeting agent that binds to an aminophospholipid on the luminal surface of intratumoral blood vessels of the vascularized tumor.

2. The method of claim 1, wherein said targeting agent binds to phosphatidylethanolamine on the luminal surface of intratumoral blood vessels of the vascularized tumor.

3. The method of claim 1, wherein said targeting agent binds to phosphatidylserine on the luminal surface of intratumoral blood vessels of the vascularized tumor.

4. A method for killing intratumoral vascular endothelial cells, comprising administering to an animal having a vascularized tumor a biologically effective amount of a binding ligand that comprises a selected therapeutic agent operatively attached to a targeting agent that binds to an aminophospholipid on the luminal surface of intratumoral vascular endothelial cells.

5. The method of claim 4, wherein said targeting agent binds to phosphatidylethanolamine on the luminal surface of intratumoral vascular endothelial cells.

6. The method of claim 4, wherein said targeting agent binds to phosphatidylserine on the luminal surface of intratumoral vascular endothelial cells.

7. A method for inducing coagulation in intratumoral vasculature, comprising administering to an animal having a vascularized tumor a vascular endothelial cell killing amount of at least a first binding ligand that comprises a selected therapeutic agent operatively attached to a targeting agent that binds to an aminophospholipid on the luminal surface of intratumoral vasculature.

8. The method of claim 7, wherein said targeting agent binds to phosphatidylethanolamine on the luminal surface of intratumoral vasculature.

9. The method of claim 7, wherein said targeting agent binds to phosphatidylserine on the luminal surface of intratumoral vasculature.

10. A method for arresting blood flow in intratumoral vasculature, comprising administering to an animal having a vascularized tumor an amount of at least a first binding ligand effective to arrest blood flow in at least a portion of the intratumoral blood vessels, the binding ligand comprising at least a first cytotoxic or coagulative agent operatively attached to a targeting agent that binds to an aminophospholipid on the luminal surface of intratumoral blood vessels of the vascularized tumor.

11. The method of claim 10, wherein said targeting agent binds to phosphatidylethanolamine on the luminal surface of intratumoral blood vessels of the vascularized tumor.

12. The method of claim 10, wherein said targeting agent binds to phosphatidylserine on the luminal surface of intratumoral blood vessels of the vascularized tumor.

13. A method for destroying intratumoral vasculature, comprising administering to an animal having a vascularized tumor an amount of at least a first binding ligand effective to collapse or destroy at least a portion of the intratumoral blood vessels, the binding ligand comprising at least a first occluding or destructive agent operatively attached to a targeting agent that binds to an aminophospholipid on the luminal surface of intratumoral blood vessels of the vascularized tumor.

14. The method of claim 13, wherein said targeting agent binds to phosphatidylethanolamine on the luminal surface of intratumoral blood vessels of the vascularized tumor.

15. The method of claim 13, wherein said targeting agent binds to phosphatidylserine on the luminal surface of intratumoral blood vessels of the vascularized tumor.

16. A method for treating an animal having a vascularized tumor, comprising administering to said animal a therapeutically effective amount of at least a first binding ligand that comprises at least a first therapeutic agent operatively attached to a targeting agent that binds to an aminophospholipid on the luminal surface of intratumoral blood vessels of a vascularized tumor.

17. The method of claim 16, wherein said targeting agent binds to phosphatidylethanolamine on the luminal surface of intratumoral blood vessels of a vascularized tumor.

18. The method of claim 16, wherein said targeting agent binds to phosphatidylserine on the luminal surface of intratumoral blood vessels of a vascularized tumor.

19. The method of claim 16, wherein said targeting agent comprises at least a first anti-aminophospholipid antibody or antigen-binding fragment thereof.

20. The method of claim 19, wherein said targeting agent comprises at least a first human antibody or antigen-binding fragment thereof.

21. The method of claim 19, wherein said targeting agent comprises at least a first IgG or IgM antibody.

22. The method of claim 19, wherein said targeting agent comprises at least a first antigen binding region of an antibody.

23. The method of claim 19, wherein said targeting agent comprises at least a first monoclonal antibody or antigen-binding fragment thereof.

24. The method of claim 23, wherein said targeting agent comprises at least a first scFv, Fv, Fab', Fab or $F(ab')_2$ fragment of a monoclonal antibody.

25. The method of claim 23, wherein said targeting agent comprises at least a first human monoclonal antibody or antigen-binding fragment thereof.

26. The method of claim 23, wherein said targeting agent comprises at least a first humanized monoclonal antibody or antigen-binding fragment thereof.

27. The method of claim 23, wherein said targeting agent comprises at least the anti-phosphatidylserine monoclonal antibody 3SB9b, or antigen-binding fragment thereof.

28. The method of claim 23, wherein said targeting agent comprises at least a first anti-aminophospholipid monoclonal antibody, or antigen-binding fragment thereof, that is prepared by a preparative process comprising:

(a) preparing an anti-aminophospholipid antibody-producing cell; and

(b) obtaining an anti-aminophospholipid monoclonal antibody from said antibody-producing cell.

29. The method of claim 28, wherein said anti-aminophospholipid antibody-producing cell is obtained from a human patient having a disease associated with the production of anti-aminophospholipid antibodies.

30. The method of claim 28, wherein said anti-aminophospholipid antibody-producing cell is obtained by stimulating a mixed population of human peripheral blood lymphocytes with an immunogenically effective amount of an aminophospholipid sample.

31. The method of claim 28, wherein said anti-aminophospholipid antibody-producing cell is obtained by immunizing an animal with an immunogenically effective amount of an aminophospholipid sample.

32. The method of claim 31, wherein said anti-aminophospholipid antibody-producing cell is obtained by immunizing an animal via intrasplenic injection of an immunogenically effective amount of an aminophospholipid sample.

33. The method of claim 31, wherein said anti-aminophospholipid antibody-producing cell is obtained by immunizing an animal by injection of an immunogenically effective amount of a *Salmonella*-coated aminophospholipid sample or an aminophospholipid micelle sample in combination with Freund's complete adjuvant.

34. The method of claim 31, wherein said anti-aminophospholipid antibody-producing cell is obtained by immunizing a transgenic mouse that comprises a human antibody library with an immunogenically effective amount of an aminophospholipid sample.

35. The method of claim 28, wherein said preparative process comprises:

(a) fusing said anti-aminophospholipid antibody-producing cell with an immortal cell to prepare a hybridoma that produces an anti-aminophospholipid monoclonal antibody; and

(b) obtaining an anti-aminophospholipid monoclonal antibody from said hybridoma.

36. The method of claim 28, wherein said preparative process comprises:

(a) immunizing an animal with an immunogenically effective amount of an aminophospholipid sample;

(b) preparing a collection of antibody-producing hybridomas from the immunized animal;

(c) selecting from the collection a hybridoma that produces an anti-aminophospholipid antibody; and

(d) culturing the selected hybridoma to provide the anti-aminophospholipid monoclonal antibody.

37. The method of claim 36, wherein the antigen binding region of the anti-aminophospholipid monoclonal antibody is operatively attached to a human antibody framework or constant region.

38. The method of claim 36, wherein the immunized animal is a transgenic mouse that comprises a human antibody library and wherein the anti-aminophospholipid monoclonal antibody is a human monoclonal antibody.

39. The method of claim 28, wherein said preparative process comprises:

(a) obtaining the anti-aminophospholipid antibody-encoding nucleic acids from said anti-aminophospholipid antibody-producing cell; and

(b) expressing said nucleic acids to obtain a recombinant anti-aminophospholipid monoclonal antibody.

40. The method of claim 28, wherein said preparative process comprises:

(a) immunizing an animal with an immunogenically effective amount of an aminophospholipid sample;

(b) preparing a combinatorial immunoglobulin phagemid library expressing RNA isolated from the spleen of the immunized animal;

(c) selecting from the phagemid library a clone that expresses an anti-aminophospholipid antibody; and

(d) expressing the anti-aminophospholipid antibody-encoding nucleic acids from said selected clone to provide a recombinant anti-aminophospholipid monoclonal antibody.

41. The method of claim 40, wherein the immunized animal is a transgenic mouse that comprises a human antibody library and wherein the recombinant anti-aminophospholipid monoclonal antibody is a recombinant human monoclonal antibody.

42. The method of claim 16, wherein said targeting agent comprises at least a first aminophospholipid binding protein or an aminophospholipid binding fragment thereof.

43. The method of claim 42, wherein said targeting agent comprises at least a first annexin or a phosphatidylserine binding fragment thereof.

44. The method of claim 43, wherein said targeting agent comprises at least a first Annexin V or a phosphatidylserine binding fragment thereof.

45. The method of claim 42, wherein said targeting agent comprises at least a first phosphatidylethanolamine binding protein or a phosphatidylethanolamine binding fragment thereof.

46. The method of claim 45, wherein said targeting agent comprises at least a first kininogen or a phosphatidylethanolamine binding fragment thereof.

47. The method of claim 16, wherein said targeting agent comprises at least two aminophospholipid binding sites.

48. The method of claim 47, wherein said targeting agent is a dimer, trimer or multimer of an anti-aminophospholipid antibody or antigen-binding fragments thereof.

49. The method of claim 16, wherein said targeting agent is prepared by recombinant expression.

50. The method of claim 16, wherein at least a second binding ligand is administered to said animal, said second binding ligand comprising a therapeutic agent or targeting agent distinct to those of said first binding ligand.

51. The method of claim 16, wherein said binding ligand comprises at least a first anticellular therapeutic agent that kills or suppresses the growth or cell division of vascular endothelial cells.

52. The method of claim 51, wherein said binding ligand comprises at least a first steroid, cytokine, antimetabolite, anthracycline, vinca alkaloid, antibiotic, alkylating agent or epipodophyllotoxin.

53. The method of claim 51, wherein said binding ligand comprises at least a first DNA synthesis inhibitor.

54. The method of claim 53, wherein said binding ligand comprises at least a first daunorubicin, doxorubicin or adriamycin.

55. The method of claim 51, wherein said binding ligand comprises at least a first cytotoxin.

56. The method of claim 55, wherein said binding ligand comprises at least a first plant-, fungus- or bacteria-derived toxin.

57. The method of claim 56, wherein said binding ligand comprises at least a first A chain toxin, bacterial endotoxin, lipid A moiety of bacterial endotoxin, ribosome inactivating protein, α-sarcin, aspergillin, restrictocin, ribonuclease, diphtheria toxin or *Pseudomonas* exotoxin.

58. The method of claim 57, wherein said binding ligand comprises at least a first ricin A chain or deglycosylated ricin A chain.

59. The method of claim 16, wherein said binding ligand comprises at least a first coagulation factor therapeutic agent.

60. The method of claim 59, wherein said binding ligand comprises at least a first human coagulation factor.

61. The method of claim 59, wherein said binding ligand comprises at least a first vitamin K-dependent coagulation factor selected from the group consisting of Factor II/IIa, Factor VII/VIIa, Factor IX/IXa and Factor X/Xa.

62. The method of claim 59, wherein said binding ligand comprises at least a first vitamin K-dependent coagulation factor that lacks the Gla modification.

63. The method of claim 59, wherein said binding ligand comprises at least a first coagulation factor selected from the group consisting of Russell's viper venom Factor X activator, thromboxane A_2, thromboxane A_2 synthase and α2-antiplasmin.

64. The method of claim 59, wherein said binding ligand comprises at least a first Tissue Factor or Tissue Factor derivative.

65. The method of claim 64, wherein said binding ligand comprises at least a first mutant Tissue Factor deficient in the ability to activate Factor VII.

66. The method of claim 64, wherein said binding ligand comprises at least a first truncated Tissue Factor.

67. The method of claim 64, wherein said binding ligand comprises at least a first dimeric or polymeric Tissue Factor.

68. The method of claim 16, wherein said binding ligand comprises at least two therapeutic agents.

69. The method of claim 68, wherein said binding ligand comprises at least a first cytotoxic agent and at least a first coagulation factor.

70. The method of claim 68, wherein said binding ligand comprises at least two therapeutic agents operatively attached to a targeting agent comprising a single aminophospholipid binding site.

71. The method of claim 68, wherein said binding ligand comprises at least two therapeutic agents operatively attached to a targeting agent that comprises at least two aminophospholipid binding sites.

72. The method of claim 68, wherein said binding ligand comprises a targeting agent that has a plurality of aminophospholipid binding sites, and wherein a plurality of therapeutic agents are operatively attached to said targeting agent at regions distinct from said aminophospholipid binding sites.

73. The method of claim 16, wherein said at least a first therapeutic agent is directly attached to said targeting agent.

74. The method of claim 16, wherein said at least a first therapeutic agent is attached to said targeting agent via an antibody, or antigen binding region thereof, that binds to said therapeutic agent.

75. The method of claim 74, wherein said binding ligand is a bispecific antibody that comprises a first, targeting antibody, or antigen binding fragment thereof, that binds to an aminophospholipid; operatively attached to a second antibody, or antigen binding fragment thereof, that binds to said at least a first therapeutic agent.

76. The method of claim 16, wherein said targeting agent is attached by a covalent bond to said at least a first therapeutic agent or to an antibody, or antigen binding fragment thereof, that binds to said therapeutic agent.

77. The method of claim 76, wherein said targeting agent is attached by a chemical cross-linker to said at least a first therapeutic agent or to an antibody, or antigen binding fragment thereof, that binds to said therapeutic agent.

78. The method of claim 76, wherein said binding ligand is a fusion protein prepared by expressing a recombinant vector in a host cell, the vector comprising, in the same reading frame, a DNA segment encoding said targeting agent operatively linked to a DNA segment encoding said therapeutic agent, or an antibody, or antigen binding fragment thereof, that binds to said therapeutic agent.

79. The method of claim 16, wherein said targeting agent is attached by an avidin:biotin bridge to said at least a first therapeutic agent or to an antibody, or antigen binding fragment thereof, that binds to said therapeutic agent.

80. The method of claim 16, wherein the vasculature of said vascularized tumor is first imaged by administering to said animal a diagnostically effective amount of at least a first detectably-labeled aminophospholipid binding construct that binds to and identifies an aminophospholipid on the luminal surface of blood vessels of the vascularized tumor.

81. The method of claim 80, wherein said detectably-labeled aminophospholipid binding construct comprises a detectably-labeled anti-aminophospholipid antibody or antigen-binding fragment thereof.

82. The method of claim 80, wherein said detectably-labeled aminophospholipid binding construct comprises a detectably-labeled aminophospholipid binding protein or aminophospholipid binding fragment thereof.

83. The method of claim 80, wherein said detectably-labeled aminophospholipid binding construct comprises the X-ray detectable compound bismuth (III), gold (III), lanthanum (III) or lead (II).

84. The method of claim 80, wherein said detectably-labeled aminophospholipid binding construct comprises the radioactive ion copper^{67}, gallium^{67}, gallium^{68}, indium^{111}, indium^{113}, iodine^{123}, iodine^{125}, iodine^{131}, mercury^{197}, mercury^{203}, rhenium^{186}, rhenium^{188}, rubidium^{97}, rubidium^{103}, technetium^{99m} or yttrium^{90}.

85. The method of claim 80, wherein said detectably-labeled aminophospholipid binding construct comprises the nuclear magnetic spin-resonance isotope cobalt (II), copper (II), chromium (III), dysprosium (III), erbium (III), gadolinium (III), holmium (III), iron (II), iron (III), manganese (II), neodymium (III), nickel (II), samarium (III), terbium (III), vanadium (II) or ytterbium (III).

86. The method of claim 80, wherein said detectably-labeled aminophospholipid binding construct comprises rhodamine or fluorescein.

87. The method of claim 16, wherein an image of the tumor vasculature is first obtained by:

(a) administering to said animal a diagnostically effective amount of at least a first targeting agent-detectable agent construct that comprises a diagnostic agent operatively attached to a targeting agent that binds to an aminophospholipid on the luminal surface of blood vessels of the vascularized tumor; and

(b) detecting the targeting agent-detectable agent construct bound to an aminophospholipid on the luminal surface of tumor blood vessels, thereby obtaining an image of said tumor vasculature.

88. The method of claim 16, wherein said binding ligand is administered to said animal via intravenous administration.

89. The method of claim 16, further comprising subjecting said animal to surgery or radiotherapy.

90. The method of claim 16, further comprising administering to said animal a therapeutically effective amount of at least a first anti-cancer agent.

91. The method of claim 90, wherein said at least a first anti-cancer agent is administered to said animal simultaneously with said at least a first binding ligand.

92. The method of claim 91, wherein said at least a first anti-cancer agent and said at least a first binding ligand are administered to said animal in a single pharmaceutical composition.

93. The method of claim 90, wherein said at least a first anti-cancer agent is administered to said animal sequentially to said at least a first binding ligand.

94. The method of claim 93, wherein said at least a first anti-cancer agent is administered to said animal subsequent to the administration of said at least a first binding ligand.

95. The method of claim 90, wherein said at least a first anti-cancer agent is a chemotherapeutic agent or a radiotherapeutic agent.

96. The method of claim 90, wherein said at least a first anti-cancer agent is an anti-angiogenic agent or apoptosis-inducing agent.

97. The method of claim 90, wherein said at least a first anti-cancer agent is an antibody-therapeutic agent construct comprising an antibody, or antigen binding fragment thereof, that binds to a surface-expressed, surface-accessible or surface-localized component of a tumor cell, tumor vasculature or tumor stroma; said antibody or fragment thereof operatively linked to a therapeutic agent.

98. The method of claim 97, wherein said at least a first anti-cancer agent is an antibody-therapeutic agent construct comprising an antibody, or antigen binding fragment thereof, that binds to a cell surface antigen of a tumor cell.

99. The method of claim 98, wherein said at least a first anti-cancer agent is an antibody-therapeutic agent construct comprising an antibody, or antigen binding fragment thereof, selected from the group consisting of B3 (ATCC HB 10573), 260F9 (ATCC HB 8488), D612 (ATCC HB 9796) and KS1/4, said KS1/4 antibody obtained from a cell comprising the vector pGKC2310 (NRRL B-18356) or the vector pG2A52 (NRRL B-18357).

100. The method of claim 97, wherein said at least a first anti-cancer agent is an antibody-therapeutic agent construct comprising an antibody, or antigen binding fragment thereof, that binds to a component of tumor stroma.

101. The method of claim 100, wherein said at least a first anti-cancer agent is an antibody-therapeutic agent construct comprising an antibody, or antigen binding fragment thereof, that binds to a connective tissue component, a basement membrane component or a component of an activated platelet.

102. The method of claim 97, wherein said at least a first anti-cancer agent is an antibody-therapeutic agent construct comprising an antibody, or antigen binding fragment thereof, that binds to a surface-expressed, surface-accessible or surface-localized component of intratumoral blood vessels of a vascularized tumor.

103. The method of claim 102, wherein said at least a first anti-cancer agent is an antibody-therapeutic agent construct comprising an antibody, or antigen binding fragment thereof, that binds to a surface-expressed component of intratumoral blood vessels of a vascularized tumor.

104. The method of claim 103, wherein said at least a first anti-cancer agent is an antibody-therapeutic agent construct comprising an antibody, or antigen binding fragment thereof, that binds to an intratumoral vasculature cell surface receptor.

105. The method of claim 104, wherein said at least a first anti-cancer agent is an antibody-therapeutic agent construct comprising an antibody, or antigen binding fragment thereof, that binds to endoglin, a TGFβ receptor, E-selectin, P-selectin, VCAM-1, ICAM-1, PSMA, a

VEGF/VPF receptor, an FGF receptor, a TIE, $\alpha_v\beta_3$ integrin, pleiotropin, endosialin or an MHC Class II protein.

106. The method of claim 105, wherein said at least a first anti-cancer agent is an antibody-therapeutic agent construct comprising an antibody, or antigen binding fragment thereof, that binds to endoglin, E-selectin or VCAM-1.

107. The method of claim 102, wherein said at least a first anti-cancer agent is an antibody-therapeutic agent construct comprising an antibody, or antigen binding fragment thereof, that binds to a ligand or growth factor that binds to an intratumoral vasculature cell surface receptor.

108. The method of claim 107, wherein said at least a first anti-cancer agent is an antibody-therapeutic agent construct comprising an antibody, or antigen binding fragment thereof, that binds to VEGF/VPF, FGF, TGFβ, a ligand that binds to a TIE, a tumor-associated fibronectin isoform, scatter factor/hepatocyte growth factor (HGF), platelet factor 4 (PF4), PDGF or TIMP.

109. The method of claim 102, wherein said at least a first anti-cancer agent is an antibody-therapeutic agent construct comprising an antibody, or antigen binding fragment thereof, that binds to a ligand:receptor complex or a growth factor:receptor complex, but that does not bind to the ligand or growth factor, or to the receptor, when the ligand or growth factor or the receptor is not in the ligand:receptor or growth factor:receptor complex.

110. The method of claim 102, wherein said at least a first anti-cancer agent is an antibody-therapeutic agent construct comprising an antibody or antigen binding fragment thereof, that binds to a cytokine-inducible component of intratumoral blood vessels of a vascularized tumor.

111. The method of claim 102, wherein said at least a first anti-cancer agent is an antibody-therapeutic agent construct comprising an antibody or antigen binding fragment thereof, that binds to a coagulant-inducible component of intratumoral blood vessels of a vascularized tumor.

112. The method of claim 97, wherein said at least a first anti-cancer agent is an antibody-therapeutic agent construct comprising an anti-tumor cell, anti-tumor vasculature or anti-tumor stroma antibody, or antigen binding fragment thereof, operatively linked to a cytotoxic agent.

113. The method of claim 97, wherein said at least a first anti-cancer agent is an antibody-therapeutic agent construct comprising an anti-tumor cell, anti-tumor vasculature or anti-tumor stroma antibody, or antigen binding fragment thereof, operatively linked to a coagulation factor or an antibody, or antigen binding fragment thereof, that binds to a coagulation factor.

114. The method of claim 16, wherein said animal has a vascularized tumor of at least about medium size.

115. The method of claim 114, wherein said animal has a large vascularized tumor.

116. The method of claim 16, wherein said animal is a mouse.

117. The method of claim 16, wherein said animal is a human patient.

118. A method for treating an animal having a vascularized tumor, comprising administering to said animal at least a first pharmaceutical composition comprising an amount of at least a first binding ligand effective to specifically kill at least a portion of the intratumoral vascular endothelial cells; wherein said binding ligand comprises at least a first cytotoxic agent operatively attached to a targeting agent that binds to an aminophospholipid expressed on the luminal surface of intratumoral blood vessels of the vascularized tumor.

119. A method for treating an animal having a vascularized tumor, comprising administering to said animal at least a first pharmaceutical composition comprising an amount of at least a first binding ligand effective to promote blood coagulation specifically in the intratumoral vasculature, the binding ligand comprising at least a first cytotoxic or coagulative agent operatively attached to a targeting agent that binds to an aminophospholipid expressed on the luminal surface of intratumoral blood vessels of the vascularized tumor.

120. A method for treating an animal having a vascularized tumor, comprising administering to said animal at least a first pharmaceutical composition comprising an amount of at least a first binding ligand effective to cause specific destruction of the intratumoral vasculature, the binding ligand comprising at least a first occluding or destructive agent operatively attached to a targeting agent that binds to an aminophospholipid expressed on the luminal surface of intratumoral blood vessels of the vascularized tumor.

121. A method for treating cancer, comprising administering to an animal with a vascularized tumor at least a first pharmaceutical composition comprising an amount of at least a first binding ligand effective to induce tumor necrosis, the binding ligand comprising at least a first therapeutic agent operatively attached to a targeting agent that binds to an aminophospholipid expressed on the luminal surface of intratumoral blood vessels of the vascularized tumor.

122. The method of any one of claims 118 to 121, wherein said targeting agent binds to phosphatidylethanolamine expressed on the luminal surface of intratumoral blood vessels of the vascularized tumor.

123. The method of any one of claims 118 to 121, wherein said targeting agent binds to phosphatidylserine expressed on the luminal surface of intratumoral blood vessels of the vascularized tumor.

124. A method for treating a patient with cancer, comprising selecting a suitable patient having a vascularized tumor and administering to said patient a therapeutically effective amount of at least a first pharmaceutical composition comprising at least a first binding ligand that comprises at least a first therapeutic agent operatively attached to a targeting agent, said targeting agent binding to an aminophospholipid expressed on the luminal surface of intratumoral blood vessels of the vascularized tumor.

125. A method for treating cancer, comprising:

(a) forming an image of a vascularized tumor by administering to an animal having a vascularized tumor a diagnostically minimal amount of at least a first targeting agent-detectable agent construct that comprises a detectable agent operatively attached to a targeting agent that binds to an aminophospholipid on the luminal surface of blood vessels of the vascularized tumor, thereby forming a detectable image of the tumor vasculature; and

(b) subsequently administering to said animal a therapeutically optimized amount of at least a first targeting agent-therapeutic agent construct comprising a therapeutic agent operatively attached to a targeting agent that binds to an aminophospholipid

on the tumor blood vessel luminal surface and thereby destroys the tumor vasculature.

126. A method for treating an animal having a vascularized tumor, comprising administering to said animal a therapeutically effective amount of a pharmaceutical composition comprising at least a first construct comprising an anti-aminophospholipid antibody, or antigen binding fragment thereof, directly or indirectly linked to at least a first therapeutic agent.

127. A method for treating an animal having a vascularized tumor, comprising administering to said animal a therapeutically effective amount of a pharmaceutical composition comprising an aminophospholipid binding protein directly or indirectly linked to at least a first therapeutic agent.

128. A method for treating an animal having a vascularized tumor, comprising administering to said animal a therapeutically effective amount of a pharmaceutical composition comprising Annexin V directly or indirectly linked to at least a first therapeutic agent.

129. A binding ligand comprising a targeting agent that binds to an aminophospholipid operatively attached to at least a first cytotoxin or coagulant.

130. The binding ligand of claim 129, wherein said targeting agent binds to phosphatidylethanolamine.

131. The binding ligand of claim 129, wherein said targeting agent binds to phosphatidylserine.

132. The binding ligand of claim 129, wherein said targeting agent comprises at least a first anti-aminophospholipid antibody or antigen-binding fragment thereof.

133. The binding ligand of claim 132, wherein said targeting agent comprises at least a first human, humanized or monoclonal antibody or antigen-binding fragment thereof.

134. The binding ligand of claim 129, wherein said targeting agent comprises at least a first aminophospholipid binding protein or an aminophospholipid binding fragment thereof.

135. The binding ligand of claim 134, wherein said targeting agent comprises at least a first annexin or a phosphatidylserine binding fragment thereof.

136. The binding ligand of claim 134, wherein said targeting agent comprises at least a first phosphatidylethanolamine binding protein, kininogen or a phosphatidylethanolamine binding fragment thereof.

137. The binding ligand of claim 129, wherein said binding ligand comprises at least a first cytotoxin.

138. The binding ligand of claim 129, wherein said binding ligand comprises at least a first coagulant.

139. The binding ligand of claim 138, wherein said binding ligand comprises at least a first Tissue Factor or Tissue Factor derivative.

140. The binding ligand of claim 129, wherein said at least a first cytotoxin or coagulant is directly attached to said targeting agent.

141. The binding ligand of claim 129, wherein said at least a first cytotoxin or coagulant is indirectly attached to said targeting agent via an antibody, or antigen binding region thereof, that binds to said cytotoxin or coagulant.

142. The binding ligand of claim 129, dispersed in a pharmaceutically acceptable formulation.

143. A bispecific antibody, comprising a first antigen-binding region that binds to an aminophospholipid operatively attached to a second antigen-binding region that binds to a therapeutic agent.

144. The bispecific antibody of claim 143, comprising a first antigen-binding region that binds to phosphatidylethanolamine.

145. The bispecific antibody of claim 143, comprising a first antigen-binding region that binds to phosphatidylserine.

146. The bispecific antibody of claim 143, comprising a first antigen-binding region that binds to an aminophospholipid operatively attached to a second antigen-binding region that binds to Tissue Factor or a Tissue Factor derivative.

147. The bispecific antibody of claim 146, further comprising Tissue Factor or a Tissue Factor derivative bound to said second antigen-binding region.

148. A construct comprising an aminophospholipid binding protein, or aminophospholipid binding fragment thereof, operatively attached to a cytotoxin or coagulant.

149. An annexin conjugate, comprising Annexin V operatively attached to truncated Tissue Factor.

150. A medicament, comprising:

(a) a first pharmaceutical composition comprising a diagnostically effective amount of a targeting agent-detectable agent construct that comprises a detectable agent operatively attached to a first targeting agent that binds to an aminophospholipid; and

(b) a second pharmaceutical composition comprising a therapeutically effective amount of a targeting agent-therapeutic agent construct that comprises a

therapeutic agent operatively attached to a second targeting agent that binds to an aminophospholipid.

151. The medicament of claim 150, wherein said first or second targeting agents are anti-aminophospholipid antibodies or antigen-binding fragments thereof.

152. The medicament of claim 150, wherein said first or second targeting agents are aminophospholipid binding proteins or aminophospholipid binding fragments thereof.

153. The medicament of claim 150, wherein said first and second targeting agents are anti-aminophospholipid antibodies, or fragments thereof, obtained from the same antibody preparation or antibody-producing hybridoma.

154. The medicament of claim 150, further comprising an anti-cancer agent.

155. A kit comprising, in at least a first suitable container, a biologically effective amount of a first anti-cancer agent comprising a first targeting agent that binds to an aminophospholipid operatively attached to at least a first therapeutic agent; and a biologically effective amount of at least a second anti-cancer agent.

156. The kit of claim 155, wherein said at least a second anti-cancer agent is a chemotherapeutic agent, radiotherapeutic agent, anti-angiogenic agent or apoptosis-inducing agent.

157. The kit of claim 155, wherein said at least a second anti-cancer agent is an antibody construct comprising an antibody, or antigen binding fragment thereof, that binds to a surface-expressed, surface-accessible or surface-localized component of a tumor cell, tumor vasculature or tumor stroma; said antibody or fragment thereof operatively linked to a therapeutic agent.

158. The kit of claim 155, wherein said first and second anti-cancer agents are comprised within a single container.

159. The kit of claim 155, wherein said first and second anti-cancer agents are comprised within distinct containers.

160. A therapeutic cocktail, comprising a combined effective amount of a first anti-cancer agent and at least a second anti-cancer agent comprising a targeting agent that binds to an aminophospholipid operatively attached to at least a first therapeutic agent.

ABSTRACT

Disclosed is the surprising discovery that aminophospholipids, such as phosphatidylserine and phosphatidylethanolamine, are specific, accessible markers of the luminal surface of tumor blood vessels. The present invention thus provides targeted therapeutic conjugates and constructs that bind to aminophospholipids, and methods of specifically delivering toxins and coagulants to the aminophospholipids of tumor blood vessels, thereby inducing thrombosis and tumor regression.